IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Japanese Application of

Takashi TSURUO, et al.

Japanese Patent Application No.: 361282/1997

Japanese Patent Filing Date: December 26, 1997

for: "TELOMERASE INHIBITOR"

VERIFICATION OF TRANSLATION

Honorable Commissioner of Patents and Trademarks

Washington, D.C. 20231

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(2) that he translated the above-identified Japanese Application from Japanese to English;

(3) that the attached English translation is a true and correct translation of the above-identified Japanese Application to the best of his knowledge and belief; and

(4) that all statements made of his own knowledge are true and that all statements made on information and belief and believed to be true, and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 USC 1001, and that such false statements may jeopardize the validity of the application or any patent issuing thereof.

February 15, 2000
Date

Jun Nakagawa
Jun NAKAGAWA

PATENT OFFICE

JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy of the following application as filed with this office.

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Application Number: Patent Application No. 361282/1997

Applicant(s): JAPANESE FOUNDATION FOR CANCER RESEARCH
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Commissioner, Takeshi ISAYAMA (seal)

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[Title of the Invention] TELOMERASE INHIBITOR

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[Material Name] Abstract 1

[Proof] Required

[Document Name] SPECIFICATION

[Title of the Invention] TELOMERASE INHIBITOR

[Claims]

[Claim 1] A telomerase inhibitor characterized in that a catechin is an effective component.

[Claim 2] A telomerase inhibitor of claim 1 wherein the catechin is epigallocatechin gallate, epigallocatechin, epicatechin gallate, or epicatechin.

[Claim 3] A telomerase inhibitor of claim 1 or 2 further comprising pharmaceutically acceptable carriers or diluents.

[Claim 4] A telomerase inhibitor of either one of claims 1 to 3 wherein the catechin content is 90 to 95% by weight.

[Claim 5] A cancer prevention or anticancer agent characterized in that a catechin is an effective component.

[Claim 6] A cancer preventing or anticancer agent of claim 5 wherein the catechin is epigallocatechin gallate, epigallocatechin, epicatechin gallate, or epicatechin.

[Claim 7] A cancer prevention or anticancer agent of claim 5 or 6 further comprising pharmaceutically acceptable carriers or diluents.

[Claim 8] A cancer prevention or anticancer agent of either one of claims 5 to 7 wherein the catechin content is 90 to 95% by weight.

[Detailed Explanation of the Invention]

[0001]

[Fields to which the Invention belongs]

The present invention relates to a telomerase inhibitor which uses catechins as an effective component and is useful as a cancer prevention agent, anticancer agent, agent to delay cancer progression, or the like.

[0002]

[Prior Art]

Various surgical, internal and, radiological antineoplastic procedures, and combinations thereof have been developed for the treatment of cancer. Extensive investigations are also being done to prevent cancer or on prophylactic means to regard the progression of a cancer as much as possible so as to prevent its transition to the fatal stage. Yet in all cases, it is evident that a further understanding as to the mechanism of the genesis and progression of cancers is essential in order to develop effective methods for their prevention and treatment.

[0003]

Recently, the presence of an enzyme called telomerase has drawn attention as

a new target in cancer therapy. This telomerase, a reverse transcriptase, synthesizes the telomeric DNA (hereinafter referred to as telomere) which is involved in the stabilization of a chromosome during cell growth. This telomere is a segment composed of a linear DNA at the end of the chromosome, and the presence of the telomere stabilizes the chromosome itself and prevents aberrations caused by binding between chromosomes.

[0004]

Research has revealed that telomeres in human tissues are shortened with age, and that cells die as this shortening progresses; thus the involvement of telomeres with the aging process and cell division cycle has become clear. Furthermore, it has been reported that telomeres in cancer cells are shorter than those in surrounding normal cells, which has further drawn attention to the telomere as an interesting target in cancer research.

[0005]

Moreover, it has been reported that activity of telomerase, a telomere-synthesizing enzyme, was detected in cells of 85% of all cancers (for example, Kim, N.W. et al., Science, 266, 2011-2015, 1994; Healy, K.C., Oncol. Res., 7, 121-130, 1995; Raymond, E. et al., Biotechnology, 7, 583-591, 1996). In contrast, almost all normal somatic cells are known to be telomerase negative. On the basis of these observations, telomere and telomerase have drawn further attention as potential targets in cancer treatment.

[0006]

Meanwhile, effect of tea in the prevention and control of cancer is being studied. Yang et al. (Yang, C.S. and Wang, Z-Y., J. Natl. Cancer Inst., 85, 1038-1049, 1993) and Fujiki et al. (Fujiki, H. et al., Nutrition Reviews, 54, 67-70, 1996) describe summarized discussion as to the anticancer effect of green tea from the epidemiological point of view. Furthermore, Liao et al. (Liao, S. et al., Cancer Letter, 96, 239-243, 1995) reported that epigallocatechin gallate (EGCG) contained in tea effectively suppressed cancer cell growth in nude mice with experimentally-induced tumors. Further, Taniguchi et al. (Taniguchi, S. et al., Cancer Letters, 65, 51-54, 1992) reported that oral administration of EGCG suppressed metastasis of malignant melanoma cells. However, none of these reports described the mechanisms underlying these effects of green tea or EGCG on cancer. In particular, these reports did not mention the effects of green tea or EGCG on telomerase, or the mechanism of anticancer activity associated with telomerase.

[0007]

[Problems to be solved by the Invention]

Although the utility of a telomerase inhibitor in cancer treatment could be surmised based on the hypothesis that the inhibition of telomerase activity in a cancer cell renders its chromosome less stable thereby shortening the life span of the cancer

cell. However, there are no reports to date regarding a substance which has an effective telomerase inhibiting activity and properties satisfactory for pharmaceutical use. Nor is there a reported case in the life span of cancer cell was effectively shortened by the use of a telomerase inhibitor.

[0008]

The object of the present invention is to provide a telomerase inhibitor having telomerase inhibiting activity, which is useful in providing an understanding of not only the involvement of telomere and telomerase with cancer, but also its prevention and treatment, whereby the telomerase inhibitor has the safe and appropriate properties for pharmaceutical use.

[0009]

[Means for resolving the problems]

A telomerase inhibitor of the present invention is characterized in using or containing catechins as an effective component. A telomerase inhibitor of the present invention can be used as a reagent for use in various experiments on telomerase inhibition per se or those involving telomerase inhibition, and further as a cancer prevention agent, anticancer agent, or the like by preparing it in a pharmaceutical preparation. Further, by using a telomerase inhibitor of the present invention, the life span of cancer cells can be shortened to kill the cells, or the progression of cancer can be effectively controlled by arresting its growth and malignancy.

[0010]

[Embodiments to carry out the Invention]

Commercially available catechins, or catechins extracted and isolated from tea by a known method can be used as an effective component of a telomerase inhibitor of the present invention.

[0011]

Catechins from tea can be extracted and isolated, for example, by extracting tea leaves with hot water or a hydrophilic organic solvent, removing natural high molecule weight substances such as caffein from the extract by extraction with chloroform, then further extracting with an organic solvent such as ethyl acetate to obtain a tea-catechin mixture, and finally isolating the catechins from this mixture by column chromatography using a hydrophilic organic solvent such as acetone as the eluant.

[0012]

Examples of catechins to be used in the present invention include (-) epigallocatechin gallate (EGCG), (-) epigallocatechin (EGC), (-) epicatechin gallate (ECG), (-) epicatechin (EC) and (+)catechin. Derivatives of these catechins can also be used to the extent that efficacy of the present invention can be attained. Of these catechins, epigallocatechin and epicatechin gallate are preferable, and

epigallocatechin gallate is particularly preferable.

[0013]

A telomerase inhibitor of the present invention can be prepared by incorporating a catechin as an effective component into an appropriate solvent, if necessary. Catechins can suitably be used by incorporating the catechin into an oil-in-water (O/W) emulsion, prepared, for example by mixing the catechin with a vegetable oil, emulsifying agent (e.g., Tween or Emulgene), and ascorbic acid (antioxidant), then lyophilizing the resulting emulsion. An example of the composition before lyophilization is 5% by weight vegetable oil, emulsifying agent, or the like, 0.1% by weight antioxidant, and 5% by weight catechin. A preferable catechin content in the inhibitor is preferably 90-95% by weight. The inhibitor can be diluted for use, if necessary.

[0014]

A pharmaceutical composition which contains a catechin having telomerase inhibiting activity as an active component can appropriately be used as a cancer prevention agent or carcinostatic agent. The carcinostatic effect includes the anticancer effect by which cancer cells are directly attacked, the effect by which the progression of cancer (malignancy) is retarded, or the like. A pharmaceutical composition having a catechin as an effective component can be formulated into various forms suited for the purpose of the treatment, by a conventional method using pharmaceutically acceptable carriers, diluents, or the like. Examples of such forms of pharmaceutical compositions include solid formulations such as tablets, pills, dispersible powders, granules, capsules, and suppositories, liquid formulations such as injections, suspensions, syrups, and emulsions, and semi-solid formulations such as plasters.

[0015]

The amount of a catechin used in a pharmaceutical composition as an effective component can be , for example, 100 to 500 mg (approximately 90 to 95% by weight of the composition) for a tablet or capsule. The dosage of a catechin used in a pharmaceutical composition can conveniently be determined as a function of the intended treatment, the nature of the condition being treated, or the like. For example, the dosage for adults can be in the range of about 500 to 2000 mg per day as the amount of effective chemical component. Furthermore, the above mentioned lyophilized O/W emulsion can appropriately be used as a formulation or as a material for formulations.

[0016]

Furthermore, various additives conventionally used in formulations can be incorporated in the pharmaceutical formulation of the present invention within a range so as not to inhibit the desired action of the catechin. Moreover, a telomerase inhibitor of the present invention could be rendered even more effective in the

treatment of cancer by the co-administration of a chemotherapeutic agent (anticancer agent) to the cancer cells being attacked by the telomerase inhibitor.

[0017]

A catechin, the effective component of the telomerase inhibitor of the present invention, can be obtained from tea components, is very safe, shows an effective activity in a small amount, and is very appropriate for use as a drug, particularly for the prevention or treatment of cancers in which telomerase is expressed.

[0018]

[Examples]

The present invention will be further illustrated by the following examples. Unless otherwise stated, "%" means "% by weight."

[0019]

Example 1

Measurement of telomerase inhibiting activity in cell-free system

Telomerase inhibiting activity of EGCG, EGC, ECG, and EC (all products of Sigma) was measured according to the Telomeric Repeat Amplification Protocol (TRAP) method described by Kim et al. (Kim, N.W. et al., Science, 266, 2011-2015, 1994) as follows:

(1) Preparation of cell extract

Cells were washed with ice-cold PBS (phosphate buffered saline) and suspended again in PBS to count the cells. After centrifuging this cell suspension, the resulting cell mass was suspended in a TRAP assay buffer (10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EDTA, 0.5% CHAPS, 10% glycerine, 5 mM 2-mercaptoethanol, and 0.1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochlorine), then the suspension was quick-frozen with liquid nitrogen. After melting, the suspension was allowed to stand in an ice bath for 30 minutes for extraction, then the resulting extract was centrifuged to obtain the supernatant as a cell extract for activity measurement.

(2) Reaction and treatment after reaction

A test compound, TS primer (50 ng), 50 μM dNTPs, and T4g32 protein (Behringer-Manheim) were added to a specified volume of the cell extract (corresponding to 200 to 1000 cells), and the admixture was incubated at 20℃ for 30 minutes. After reaction, CX primer (100 ng) and an internal standard for PCR, ITAS (10^{-18} g), were added to the reaction solution, then PCR was performed in the presence of AmpliTaq DNA polymerase (2 U), i.e., (94℃, 40 seconds → 50℃, 40 seconds → 72℃, 60 seconds) x 35 cycles → 72℃, 2 minutes, to obtain PCR products.

[0020]

Base sequences of the primers used in the abovementioned procedure are as follows:

TS primer:

5'-AATCCGTCGAGCAGAGTT-3' (SEQ ID NO: 1)

CX primer:

5'-GTGCCCTTAACCCTTACCCTTACCCTAA-3' (SEQ ID NO: 2)

ITAS:

5'AATCCGTCGAGCAGAGTTGTGAATGAGGCCTTCGAGGCTCTGAAG AGAAGCACCCTGCTCAACCCCAACCAGCGGCTGCCTAAGGTGGAGATCCT GCGCAGTGCCATCCAGTACATTGAGCGCCTATTAGGGTAAGGGTAAGGGT AAGGG-3' (SEQ ID NO: 3)

In the in vitro reaction system, telomerase performs addition synthesis of telomere at the 3' end of the TS primer. Of the 24 bases at the 3'-end side of the CX primer, 21 bases are identical to a telomere sequence, hence the telomere product can be amplified by PCR with a combination of TS primer and CX primer. The 5' end part and the 3' end part of the ITAS sequence are made identical to the TS primer sequence and the CX primer sequence, respectively.

(3) Detection and quantitative analysis of reaction products

The PCR products obtained in the abovementioned procedure were resolved by polyacrylamide gel electrophoresis and the resulting DNA bands stained with SYBR Green (Takara Shuzo) were detected by irradiation with a UV transilluminator. The detected bands were photographed. The intensity of each band was quantitatively analyzed using the NIH Image 1.60 software program. Telomerase activity was expressed as the intensity of a sample band relative to that of the band corresponding to the ITAS fragment (control).

[0021]

Results are shown in Figure 1. From Figure 1, it is evident that EGCG has the highest inhibiting activity among the five catechins.

[0022]

Example 2

Telomerase inhibiting activity of EGCG

Telomerase activity in reaction solutions of 8 different EGCG concentrations ranging from 0.1 to 20 μM was measured in the same manner as described in Example 1 except that a primer, PS, was used at two different concentrations. Results are shown in Figure 2. A Dixon plot was plotted from the results in Figure 2, which gave a Ki value of about 100 nM (see Figures 2 and 3).

[0023]

Example 3

Telomerase inhibiting activity in cells

The telomerase inhibiting activity of EGCG was assayed in monoblastoid leukemia U937 cells (American Type Culture Collection, ATCC) and colon adenocarcinoma HT29 cells.

[0024]

An RPMI1640 medium (Nissui) supplemented with 10% fetal bovine serum (FBS) was used for U937 cells, and an RPMI1640 medium supplemented with 5% FBS and 5% fetal calf serum (FCS) was used for HT29 cells.

[0025]

The cells were incubated in a 15 μM EGCG solution for 2 hours and washed in PBS. The washed cells were suspended in an RPMI1640 medium (without serum) at a concentration of 2 x 10^6 cells/ml, then Streptolysin O (Sigma, 5 U/ml), TS primer (10 μM), spermidine (1 mM), and Imipramine (50 μM) were added, and the admixture was incubated at room temperature for 10 minutes. Streptolysin O is a substance which increases the permeability of a substance through the cell membrane, and enables the TS primers and other substances to be incorporated into the cell.

[0026]

The permeabilization reaction was stopped by adding an equal volume of RPMI1640 medium supplemented with 10% FBS, after which the cell suspension was further incubated for 1 hour to seal the cell membrane. The cells thus obtained, in which TS primer and other substances were incorporated, were incubated at room temperature for 1 hour to coat the cell membrane, and at the same time, to allow the intracellular telomerase reaction to progress. The cells were then washed twice with PBS, pelleted, and frozen for a TRAP assay to detect telomerase products.

[0027]

Namely, the cells were washed with ice-cold PBS and suspended again in PBS to count the cells. This suspension was centrifuged, the resulting cell mass was suspended in a TRAP assay buffer, then the suspension was quick-frozen with liquid nitrogen. After melting, the suspension was allowed to stand in an ice bath for 30 minutes, then the resulting extract was centrifuged to obtain the supernatant as a cell extract. TS primer (344 nM), ACX primer (385 nM), 50 μM dNTPs, T4g32 protein (Behringer-Manheim), an internal standard for PCR, TSNT (0.02 pM), and NT primer (385 nM) were added to a specified volume of the cell extract (corresponding to 200 to 1000 cells), then PCR was performed in the presence of Ampli Taq DNA polymerase (2 U), i.e., (94℃, 40 seconds → 50℃, 40 seconds → 72℃, 60 seconds) x 35 cycles → 72℃, 2 minutes, to obtain PCR products.

Base sequences of the primers used in this reaction are as follows.

ACX primer:

5'-GCGCGGCTTACCCTTACCCTTACCCTAACC-3' (SEQ ID NO: 4)

TSNT:

5'-AATCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT-3' (SEQ ID: NO. 5)

NT primer:

5'-ATCGCTTCTCGGCCTTTT-3' (SEQ ID NO: 6)

[0028]

The telomerase products thus obtained were resolved by polyacrylamide gel electrophoresis, after which DNA bands were stained with SYBR Green (Takara Shuzo) and detected by irradiation with a UV transilluminator. The intensity of each band was quantitatively analyzed using the NIH Image 1.60 software program and telomerase activity was expressed as the intensity of a sample band relative to that of the band corresponding to the ITAS fragment (control).

[0029]

In the abovementioned PCR, a part elongated by telomerase was amplified using TS primer as the forward primer and ACX primer as the return primer. Further, the 36-bp internal standard (TSNT) and its own return primer (NT) were used as described by Kim et al. (Kim, N.W. and Wu, F., Nucleic Acids Res., 25, 2595-2597, 1997).

[0030]

Results are shown in Figure 4. The results in Fig. 4 showed that the treatment of the cells with EGCG markedly inhibited intracellular telomerase activity. Minor signals were also detected in the absence of TS primer. These signals were deemed to be derived from endogenous telomere sequences, or the like, and was similarly inhibited by the EGCG treatment.

[0031]

Example 4

Mechanism of effect of EGCG on telomerase

The cells were treated with 15 μM EGCG for 2 hours and washed with ice-cold PBS. The cell mass obtained by centrifugation was suspended in a TRAP assay buffer and the resulting suspension was quick-frozen with liquid nitrogen. After melting, the suspension was allowed to stand in an ice bath for 30 minutes, then the resulting extract was centrifuged to obtain the supernatant as a cell extract. A test compound, TS primer (50 ng), 50 μM dNTPs, and T4g32 protein (Behringer-Manheim) were added to a specified volume of the cell extract (corresponding to 200 to 1000 cells), and the admixture was incubated at 20℃ for 30 minutes. After reaction, CX primer (100 ng) was added to the reaction solution, then PCR was performed in the presence of Ampli Taq DNA polymerase (2 U) and ITAS (10^{-18} g), i.e., (94℃, 40 seconds → 50℃, 40 seconds → 72℃, 60 seconds) x 35 cycles → 72℃, 2 minutes, to obtain PCR products. The PCR products thus obtained were resolved by polyacrylamide gel electrophoresis and the resulting DNA bands stained with SYBR Green (Takara Shuzo) were detected by irradiation with a UV transilluminator. The intensity of each band was quantitatively analyzed using the NIH Image 1.60 software program. Telomerase activity was expressed as the intensity of a sample band relative to that of the band corresponding to the ITAS fragment.

[0032]

The abovementioned procedure was carried out for both U937 cells and HT29 cells. Results showed that the EGCG treatment of cells had no effect on telomerase activity, when tested in vitro because of the dilution effect during the solubilization of cells. This observation along with the results of Examples 1 to 3 revealed that EGCG directly and reversibly inhibited telomerase activity in the cells, and that this inhibition was not through the intracellular information transfer system (intracellular signaling pathway).

[0033]

Example 5

U937 cells and HT29 cells were each subcultured on a medium with and without EGCG to study the effect of addition of EGCG on cell growth. The medium used was the same as used in Example 3.

[0034]

Five subculture lines for U937 cells and nine subculture lines for HT29 cells were prepared. The first culture of each line was made by inoculating cells of each stock culture at a density of 5×10^5 cells per 10-cm dish. For each subculture, two lines were cultured on a medium without EGCG (control lines) and the rest of the lines were cultured on a medium supplemented with 15 μM EGCG.

[0035]

Cells were passaged, sampled and counted every four days. The incubation medium was freshly changed every day.

[0036]

Results are shown in Figure 5 (U937 cells) and Figure 6 (HT29 cells). Symbol ○ shows the control line cultured on a medium without EGCG. In Figure 5, symbols ●, ▲, and ■ show the lines cultured on a medium supplemented with 15 μM EGCG. Cells of all the lines entered cell crisis at day 53 (●), day 44 (▲), and day 29 (■), and thereafter showed morphological changes similar to cell senescence. In Figure 6, both symbols ▲ and ● show the lines cultured on a medium supplemented with 15 μM EGCG. Cells of both lines entered cell crisis at day 75 (▲) and day 57 (●), and thereafter showed morphological changes similar to cell senescence.

[0037]

U937 cells and HT29 cells entered crisis approximately at PD25 and PD60, respectively, showing characteristic morphological changes (rounding up and detachment for HT29 cells and raggedness of the plasma membrane for U937 cells). U937 cells died at PD40 and HT 29 cells died at PD70. PD is the number of cycles in which the cell population doubles during cell growth and is calculated by monitoring cell counts using a hemocytometer.

[0038]

The form of the cells did not revert and was maintained when the cells with morphological changes were transferred and cultured on a medium without EGCG, which confirmed that the morphological changes were not temporary.

[0039]

HT 29 cells from PD57 to PD60 in cell crisis were pooled and the genomic DNA was prepared by a salting-out/ethanol precipitation method (Stratagene). Further, integrity of the isolated genomic DNA was confirmed by gel electrophoresis (ethidium bromide staining).

[0040]

The isolated genomic DNA (10 μg) was digested with HinfI and RsaI and the resulting digests were subjected to Southern blotting by the ordinary method, after which DNA fragments were detected using a TeloQuant assay kit (PharmMingen).

[0041]

Results are shown in Figure 7. While cleavage sites recognized by restriction enzymes HinfI and RsaI are numerous in a normal genomic DNA, such cleavage sites do not exist in the subtelomere or telomere region. Accordingly, if a genomic DNA is digested with the abovementioned restriction enzymes, only the telomere region would remain undigested. This DNA fragment is called the Terminal Restriction Fragment (TRF) which can be detected by Southern blotting to analyze telomere lengths. TRFs of EGCG-treated or untreated HT29 cells obtained as mentioned above were detected using a TeloQuant assay kit. Figure 7 shows the results of densitometric analysis of the detected signals. It was observed that TRFs in the cells which entered crisis due to the EGCG treatment (●) were shortened by about 1.1 kb as compared to those in the untreated cells(○). Further, it was confirmed that successive EGCG treatment of HT29 cells caused telomere diminishment.

[0042]

Further, analysis of DNA content of cells before entering crisis by the ordinary method using flowcytometry revealed a 50 to 100% and a 10 to 20% increase in the aneuploidy and cell cycle G2/M fractions, respectively, for both U937 cells and HT29 cells.

[0043]

In conclusion, it is evident from these results that cell crisis to death was caused by intracellular telomerase inhibition by EGCG.

[0044]

[Effects of the Invention]

Catechins used in the present invention have unconventionally strong telomerase inhibiting activity, and can provide a telomerase inhibitor which is useful in providing an understanding of the involvement of telomerase with a cancer, and its prevention and treatment, and which possesses the safe and appropriate properties for

pharmaceutical use.

[0045]

[Sequence Listing]

SEQ ID NO: 1
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 18 bases
STRANDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
PROPERTIES: TS primer for PCR
SEQUENCE:
AATCCGTCGA GCAGAGTT 18

SEQ ID NO: 2
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 28 bases
STRANDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
PROPERTIES: CX primer for PCR
SEQUENCE:
GTGCCCTTAA CCCTTACCCT TACCCTAA

SEQ ID NO: 3
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 150 bases
STRANDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
PROPERTIES: ITAS internal standard for PCR
SEQUENCE:
AATCCGTCGA GCAGAGTTGT GAATGAGGCC TTCGAGGCTC 40
TGAAGAGAAG CACCCTGCTC AACCCCAACC AGCGGCTGCC 80
TAAGGTGGAG ATCCTGCGCA GTGCCATCCA GTACATTGAG 120
CGCCTATTAG GGTAAGGGTA AGGGTAAGGG 150

SEQ ID NO: 4
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 30 bases
STRANDNESS: Single

TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
PROPERTIES: ACX primer for PCR
SEQUENCE:
GCGCGGCTTA CCCTTACCCT TACCCTAACC 30

SEQ ID NO: 5
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 36 bases
STRANDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
PROPERTIES: TSNT internal standard for PCR
SEQUENCE:
AATCCGTCGA GCAGAGTTAA AAGGCCGAGA AGCGAT

SEQ ID NO: 6
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 18 bases
STRANDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
PROPERTIES: NT primer for PCR
SEQUENCE:
ATCGCTTCTC GGCCTTTT 18

[Brief Description of the Drawings]

[Figure 1]

Figure shows the telomerase activity of different compounds.

[Figure 2]

Figure shows changes in telomerase activity as a function of EGCG concentration.

[Figure 3]

Figure shows a Dixon plot for the telomerase inhibiting activity of EGCG.

[Figure 4]

Figure shows the telomerase inhibiting activity of EGCG in a cell.

[Figure 5]

Figure shows the effect of EGCG on cell growth.

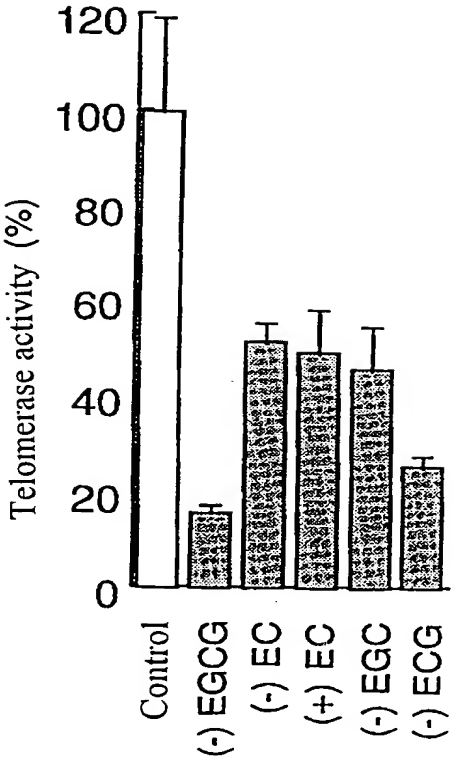
[Figure 6]

Figure shows the effect of EGCG on cell growth.

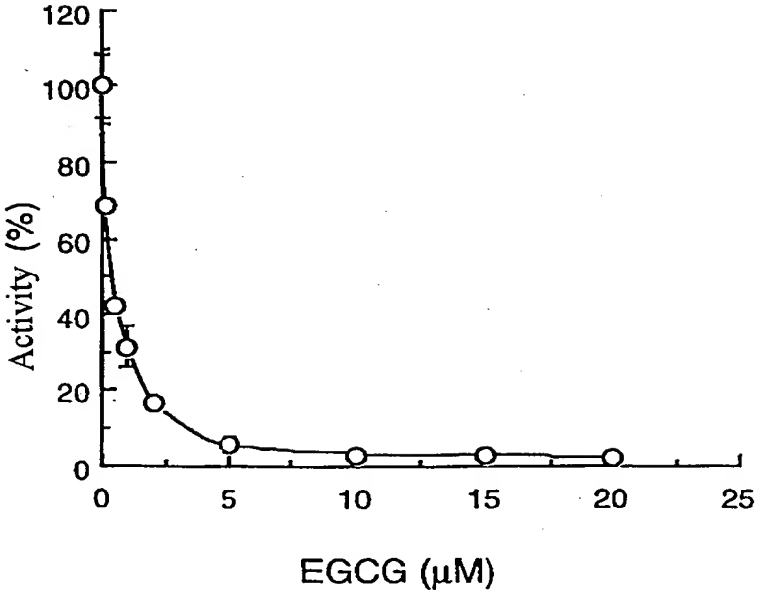
[Figure 7]

Figure shows the distribution of TRF lengths from HT29 cells at PD67 to PD70 in subcultures in the presence or absence of EGCG (15 μM).

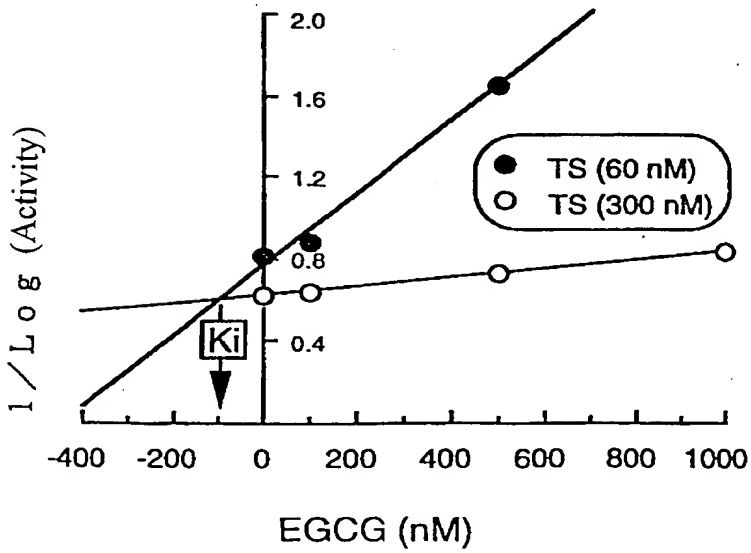
[Document Name] Drawings
[Figure 1]



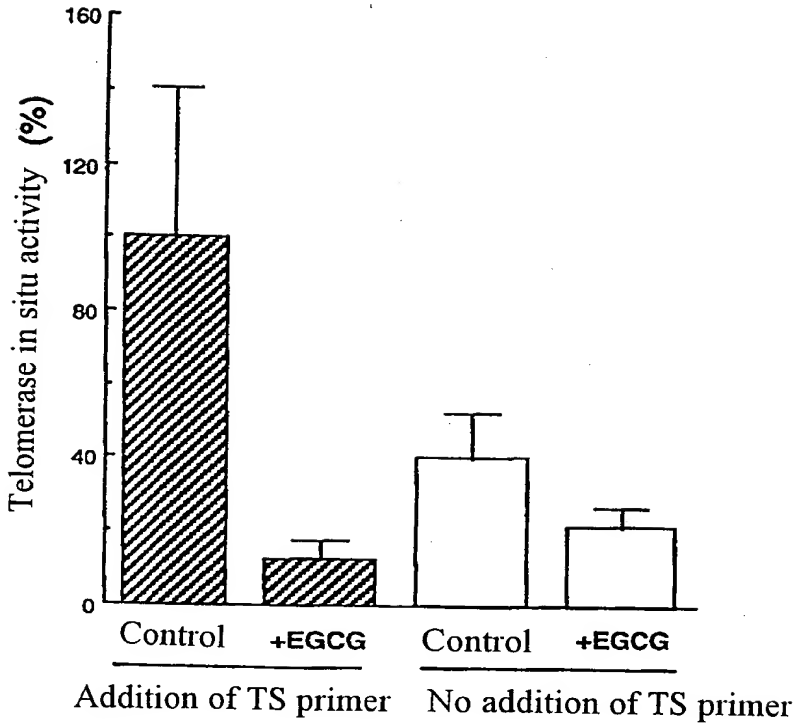
[Figure 2]



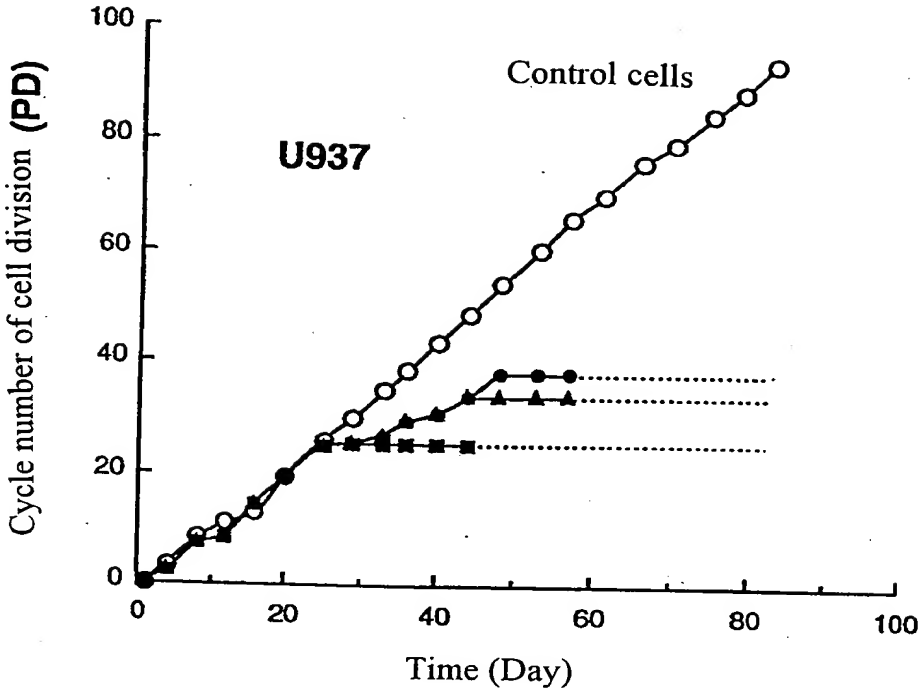
[Figure 3]



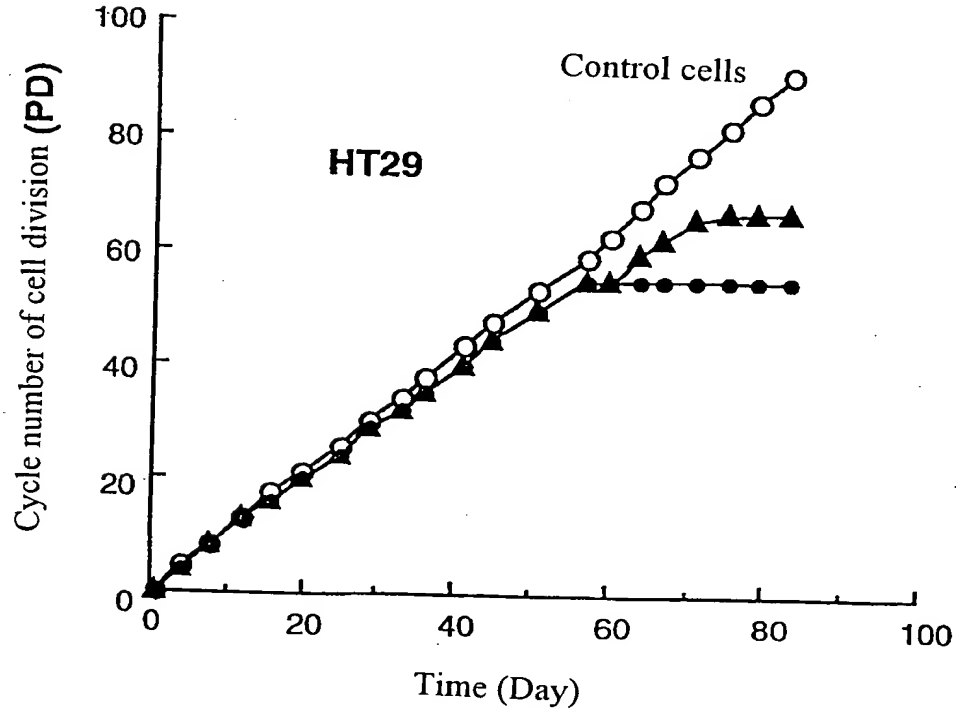
[Figure 4]


160
120
80
40
0
Telomerase in situ activity (%)
Control
+EGCG
Control
+EGCG
Addition of TS primer
No addition of TS primer

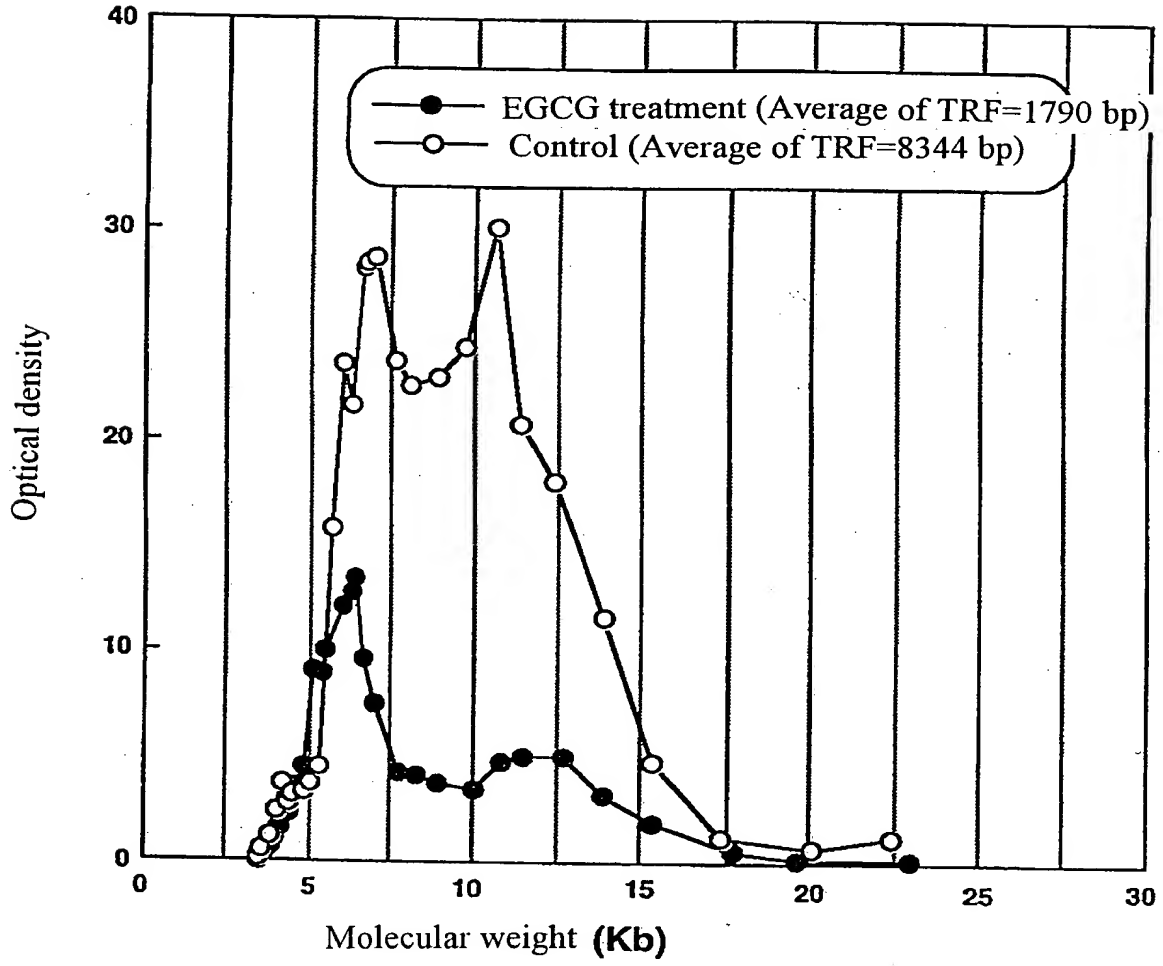
[Figure 5]



[Figure 6]



[Figure 7]


EGCG treatment (Average of TRF=1790 bp)
Control (Average of TRF=8344 bp)
Optical density
Molecular weight (Kb)
0
10
20
30
40
0
5
10
15
20
25
30

Abstract

[Abstract]

[Objects] The object is to provide a telomerase inhibitor which has telomerase inhibiting activity useful in providing an understanding of the involvement of telomerase with a cancer, and its prevention and treatment, and which possesses the safe and appropriate properties for pharmaceutical use.

[Means for achieving the object] A telomerase inhibitor is prepared by using catechins, in particular epigallocatechin gallate as an effective component.

[Selection of the drawing] Fig. 1